ENVIRONMENTAL ASSESSMENT

PHASE I TESTING OF LIVE-ATTENUATED DENGUE VACCINE CANDIDATES PRODUCED BY THE MAHIDOL UNIVERSITY AND BY THE WALTER REED ARMY INSTITUTE OF RESEARCH

Prepared For:
U.S. Army Medical Research, Development, Acquisition and Logistics Command (Provisional)
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EXECUTIVE SUMMARY

This Environmental Assessment (EA), *Phase I Testing of Live-Attenuated Dengue Vaccine Candidates Produced by the Mahidol University and by the Walter Reed Army Institute of Research*, was researched and prepared by the Walter Reed Institute of Research (WRAIR) for the U.S. Army Medical Research, Development, Acquisition and Logistics Command (USAMRDALC) (Provisional).

The proposed series of clinical studies will give live-attenuated (weakened) dengue virus vaccines to volunteers to induce protective antibodies and confirm the absence of side effects. Candidate dengue vaccines will be selected only after careful evaluation of safety in small inpatient clinical trials. Volunteers will be followed daily as outpatients.

This EA was prepared in accordance with guidance provided in Army Regulation 200-2 (Environmental Effects of Army Actions) and the National Environmental Policy Act (NEPA) (42 USC 4321-4347).

The significant environmental consequences of the proposed action are minimal. Risk of transmission of live-attenuated dengue vaccine virus to others is extremely small. Alternatives to the proposed action include the admission of all volunteers to a closed inpatient ward for part or all of the trial and the no action alternative.

DESCRIPTION OF THE ACTION

The objective of this EA is to evaluate the environmental impacts associated with the conduct of human use clinical trials using live-attenuated dengue viruses by the Department of Virus Diseases at the WRAIR. The studies will be performed under the Infectious Disease Research Program (IDRP) which is authorized and funded through Congress and implemented through the Department of Defense (DoD) by the Army. The mission of the IDRP is to preserve soldier performance by the prevention of infectious disease (e.g., dengue fever).

The proposed outpatient clinical trials involve the use of live-attenuated (weakened) dengue viruses. These are viruses that have been deliberately weakened in the laboratory and tested extensively for failure to resemble the original dengue viruses that caused illness. Live-attenuated dengue vaccines scheduled for outpatient trials have been previously tested for safety and attenuation in small controlled clinical trials. Inpatient volunteers have experienced mild erythema and tenderness of the injection site, and sometimes mild illness with headache and rash, but no severe or serious reactions with these vaccines.

Because a few volunteers may have vaccine virus present in their blood following vaccination, there is a remote possibility that they may spread the virus to another person if a mosquito feeds on them and then on another person. To eliminate this possibility, the 30 volunteers participating in the study will be observed during the time when virus may be present in their blood (days 8-12 after immunization). A local motel will be provided as a mosquito-controlled environment during this time (a total of four days and nights). Volunteers will be informed that there is low risk for transmission of virus to others through mosquito bites during this period, that external contacts are prohibited, and given insect repellents for use to avoid mosquito bites. The housing area will be carefully checked for mosquitos by experienced entomologists, and clinical staff will remain available on-site. Blood specimens taken from the volunteers will be routinely screened for dengue virus.

During the period of viremia (days 8-12 after immunization), volunteers will be released from the motel only for emergencies, and with safety precautions. These measures include the provision to the volunteer of a card with contact telephone numbers of trial principal investigators; a letter indicating their participation in a clinical trial of dengue vaccine; and notification of local medical personnel as required. In addition, volunteers will be given insect repellents and required to indicate understanding of the need for their use.

External contacts will be permitted during the rest of the trial (days 1-7 and days 13-21 after immunization). The risks for external contacts to acquire dengue when volunteers are not viremic are nil because there is no transmission without virus in the blood.

The modified protocol has been approved by the WRAIR Ad-Hoc Scientific Review Committee and the WRAIR Human Use Committee. The original protocol was approved for implementation by the United States Army Medical Research Institute for Infectious

Diseases (USAMRIID) Scientific and Human Use Review Committees and the Human Subjects Research Review Board (HURRAO Log #A-5618).

The studies will be performed in compliance with the requirements of other Federal agencies (40 CFR 1505.25).

PURPOSE AND NEED FOR THE ACTION

These studies involve Phase I clinical trials in outpatient volunteers vaccinated with live-attenuated dengue virus vaccines. Information collected from these trials will be furnished to the Food and Drug Administration (FDA) for incorporation into vaccine Investigational New Drug (IND) permits. If found safe, these vaccines may be considered for further expanded testing in volunteers.

These trials may provide evidence to support the feasibility of live-attenuated dengue vaccines. No dengue virus vaccine is currently available. Dengue fever is prevalent in tropical climates. Development of a dengue vaccine will significantly contribute to the national defense posture by protecting U.S. military personnel stationed in areas of the world where this disease is endemic.

ALTERNATIVES CONSIDERED

The alternatives include hospitalizing volunteers for all or part of the proposed trial (Alternative I) and no action (Alternative II). Alternative I would incur negligible risk of transmission of dengue virus to others but at increased cost in time to the volunteers and expenses for the trial. Hospitalization does not significantly reduce risks to the volunteers and would involve extended separation from external contacts.

The no action alternative (Alternative II) would be not to perform the clinical trials. Implementation of the no action alternative would forfeit the contribution of these studies to the prevention of disease of military importance.

AFFECTED ENVIRONMENT

The study will be located at the Clinical Trials Unit, Building 40, WRAIR, Washington, D.C. 20307-5100. Volunteers will be immunized and then examined daily at this outpatient facility for three weeks. For four days and nights, on days 8-12 after immunization, volunteers will be housed at the Econo Lodge located in Silver Spring, Maryland located near the Walter Reed Army Medical Center (WRAMC). The inn is a commercial residential facility with 16 rooms under contract with the WRAMC. Detailed descriptions of the baseline environment where the studies will take place are provided in

other recent NEPA documentation (WRAIR, 1993a; WRAIR, 1993b; Rogers, Golden & Halpern, 1990).

The areas surrounding the study environment are at low risk for mosquito breeding. They are not conducive to the transmission of weakened dengue vaccine viruses. The controlled environment created under the proposed action would be made essentially mosquito-free through barrier screening of quarters, surveillance for the insects, and application of acceptable insecticides as needed.

ENVIRONMENTAL CONSEQUENCES

Potential environmental consequences of the proposed action are minimal. In order for another person to be infected by the attenuated dengue vaccine virus during these trials, a series of unlikely events must occur. The risks associated with each of these events are discussed in detail below. The likelihood of all three biologic events occurring simultaneously (i.e., having a volunteer with vaccine virus in his/her blood stream, with that virus being able to infect mosquitos, and having an infectable type of mosquito present) is very low.

Dengue virus is a mosquito-borne virus. That is, for dengue virus to transmit from a vaccinated volunteer to a second person, three separate biologic events must occur. First, the volunteer must have enough vaccine virus in his/her blood stream so that mosquitos feeding on the volunteer ingest an infective dose. Second, vaccine virus must be able to infect mosquitos, which is required for the virus to become capable of infecting another person. Third, the proper type of mosquito must feed on the viremic volunteer; only two species of mosquitos (*Aedes aegypti* and *Aedes albopictus*) are capable of being infected with, and transmitting, dengue virus.

The probability of live-attenuated vaccine virus entering the blood after vaccination is less than that expected following infection with normal (unweakened) infective virus. Appendix A provides documentation of decreased frequency and degree of viremia in recipients of live-attenuated dengue vaccines.

Live-attenuated vaccine viruses do not infect mosquitos as efficiently as normal infective virus. Several study vaccines have been tested for their ability to infect mosquitos, and shown to be less efficient in transmitting infection compared to unweakened viruses. The passage of virus from vaccinated volunteers to others is reduced dramatically if mosquitos fail to transmit infection. Appendix B lists the data for ineffective transmission of live-attenuated dengue vaccine virus in mosquitos.

In addition, only certain mosquito types (*Aedes aegypti* or *Aedes albopictus* species) are capable of transmitting dengue virus. Entomologists at the WRAMC and the WRAIR have surveyed the area around the post for these mosquito types repeatedly and

failed to detect any potentially injectable mosquitos. Appendix C shows that these types of mosquitos are not found in the Washington, D.C. area even during warm summer months.

In order to reduce transmission risk further, environmental barriers will be placed which have proven effective in reducing exposure to mosquito bites (i.e., use of insect repellents, entomological monitoring for mosquitos). In addition, volunteers will be under direct observation by clinical staff during the period of maximal risk. Consequently, the risks to the environment and human health associated with these trials are negligible.

REFERENCES

Rogers, Golden & Halpern, Inc. 1990. Environmental Assessment Revised Master Plan Forest Glen Section Walter Reed Army Medical Center, September 26, 1990.

Walter Reed Army Institute of Research. 1993a. Walter Reed Army Institute of Research (WRAIR) Leased Facilities Environmental Assessment.

Walter Reed Army Institute of Research. 1993b. Walter Reed Army Institute of Research (WRAIR) Environmental Assessment.

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APPENDIX A. SUMMARY OF VIREMIA DETECTED IN DEN 1 AND DEN 4 VACCINE RECIPIENTS

Virus is detectable in the blood on day 8-12 of illness in classical dengue fever. In vaccine volunteers, attempts to detect viremia are conducted every two days over 21 days following inoculation. The results summarize the results of the trials to date. These indicate that the frequency, duration, and amount of viremia are decreased in recipients of attenuated vaccine virus compared to historical controls. In addition, all vaccine candidates have been shown to cause decreased viremia in other primates.

Table 1. Frequency and Intensity of Viremia in Vaccine Volunteers

Vaccine	Dose Vaccine (pfu)	No. Volunteers	# with Viremia (%)	Day of Viremia[a]	Amount Viremia[b] (pfu/ml)
Den 1 Strain 16007 PDK 10 (WRAIR) Vaccine	2×10^6	3	1 (33)	10	1560
Den 1 Strain 16007 PDK 10 (Mahidol) Vaccine	$0.4\text{-}1.2 \times 10^5$	7	1 (14)	day 7	low
Den 1 Strain 16007 PDK 10 (Mahidol) Vaccine	300-17000 (dose-ranging study)	12	4 (25)	day 8 (2 vols.), 9, 11	low
Den 4 Strain 1036 PDK 48 Vaccine	$1\text{-}2 \times 10^4$	5	3 (60)	day 8, 9, 11	low
Den 4 Strain 1036 PDK 48 Vaccine	15-3700 (dose-ranging study)	12	2 (16)	not available	low

a: days counted from the day of inoculation (day 0)
b: low titer virus recovered from delayed virus isolation; no virus recovered directly from sera

APPENDIX B. TRANSMISSION OF DENGUE VACCINE VIRUS IN MOSQUITOS

In the unlikely event of a potential vector mosquito feeding on a viremic volunteer, the likelihood of the mosquito transmitting the virus to another human host has been shown to be extremely low. Virus transmission into droplets was also decreased with vaccine viruses. The combination of low level human viremia (virus was grown up to high titers in these experiments), reduced dissemination to salivary glands in vector mosquitos, and reduced release by infected vectors makes it highly unlikely the dengue vaccine viruses will be transmitted by mosquito in open trials.

Table 1. Summary Results for Oral infection of *Aedes aegypti* Mosquitos with Mahidol DEN 1 and 4 Vaccines

	PARENT	PDK13
spread to salivary gland	definite	reduced
replication in mosquito	moderate	reduced
transmission	demonstrated	much reduced
attenuation	-	retained

Data presented at the Eighth WHO Peer Review Meeting on Dengue Vaccine Development, 29-30 September 1990. Center for Vaccine Development, Mahidol University, Bangkok, Thailand.

Transmission of Dengue 1 (16007 PDK 13) Vaccine Virus in the Environment

Aedes aegpyti mosquitos were fed on a mixture of guinea pig erythrocytes, sucrose solution, and virus (dengue 1 parent virus, dengue 1 PDK 13 vaccine virus, and isolates from two recipients of dengue 1 PDK 13). All four viruses infected the mosquitos but the vaccine strain and the 2 isolates were less likely to disseminate to the salivary glands (see Table 2).

Transmission was demonstrated by having infected mosquitos feed on blood droplets. Virus released while probing into the droplets was detected by inoculation of the droplet into *Toxorhynchites splendens* mosquitos. It was found that virus release occurred from 36% of mosquitos infected with parent virus but only 13% of those infected with dengue 1 PDK 13 and none of those infected with the isolates from the vaccine recipients.

Table 2. Dengue 1 Strain 16007 Vaccine Virus Transmission in Mosquitos

Virus	Titer of Blood Meal (Log10/ml)	% Mosquitos Orally Infected	% Dissemination	% Mosquitos Transmitting Virus
Parental 16007	8.5	60 (105/174)	71 (75/105)	36 (5/14)
PDK 13 Vaccine	7.6	50 (98/183)	21 (38/182)	13 (2/16)
Volunteer 1b1	7.3	50 (99/200)	25 (49/200)	0 (0/1)
Volunteer 1b10	6.9	40 (110/256)	18 (46/256)	0 (0/4)

Data presented at the Seventh WHO Peer Review Meeting on Dengue Vaccine Development, 7-11 August 1989. Center for Vaccine Development, Mahidol University, Bangkok, Thailand.

Transmission of Dengue 4 (1036 PDK 48) Vaccine Virus in the Environment

Aedes aegypti mosquitos were fed on a mixture of guinea pig erythrocytes, sucrose solution, and virus suspensions collected from LLC-MK2 and C6/36 cells of *Toxorhynchities splendens* mosquitos. Only two of the four feeding suspensions of virus (obtained from C6/36 cells) infected the mosquitos (see Table 3).

None of the mosquitos that were successfully orally infected by vaccine virus had dissemination of the virus to the salivary glands. Transmission was thus aborted by the attenuation of this vaccine strain.

Table 3. Dengue 4 Strain 1036 PDK 48 Vaccine Virus in Mosquitos

Feeding Suspension	Titer of Blood Meal (Log10/ml)	% Mosquitos Orally Infected	% Dissemination	% Mosquitos Transmitting Virus
LLC-MK	7.7	0 (0/38)	0 (0/58)	-
C6/36	7.7	1 (1/81)	0 (0/81)	-
C6/36	8.8	5 (2/41)	0 (0/41)	-
Toxo	7.4	0 (0/82)	0 (0/82)	-

Data presented at the Ninth WHO Peer Review Meeting on Dengue Vaccine Development, 26-29 August 1991. WHO Collaborating Centre for Research on the Immunopathology of Dengue Haemorrhagic Fever, Bangkok, Thailand.

APPENDIX C. SUMMARY OF MOSQUITO COLLECTIONS IN THE WASHINGTON, D.C. AREA[a]

The two mosquito vectors for dengue fever are *Aedes aegypti* and *Aedes albopictus*. There were no collections of these mosquitos in the Washington, D.C. area during 1991-1993. Additionally, no cases of mosquito-borne diseases were reported on military installations in the area.

Installation & State (Year)	Total No. of Specimens[b]	*Aedes aegypti* (%)	*Aedes albopictus* (%)
Bolling AFB, DC (1991)	136	0 (0)	0 (0)
Andrews AFB, MD (1991)	59	0 (0)	0 (0)
Bolling AFB, DC (1992)	-[c]	0 (0)	0 (0)
Aberdeen PG, MD (1993)	516 adults; 6 larvae; 133 adults reared from eggs	0 (0)	0 (0)
Ft. A.P. Hill (1993)	527 adults; 0 larvae; 52 adults reared from eggs	0 (0)	0 (0)
Ft. Belvoir (1993)	9 adults; 0 larvae; 49 adults reared from eggs	0 (0)	0 (0)
Ft. Meade, MD (1993)	276 adults; 0 larvae; 44 adults reared from eggs	0 (0)	0 (0)
WRAMC, DC (1993)	0 adults; 37 larvae; 27 adults reared from eggs	0 (0)	0 (0)
WRAMC, MD (Forest Glen Annex) (1993)	0 adults; 0 larvae; 65 adults reared from eggs	0 (0)	0 (0)

[a] Data summarized from:

1. Pest Identification Study No. 16-61-AZUF-93, "Mosquito Surveillance Within the U.S. Army Environmental Hygiene Activity - North Support Area, 1993", U.S. Army Environmental Hygiene Agency, Ft. George G. Meade, MD 20755-5225. The U.S. Army Environmental Hygiene Activity (USAEHA) - North surveillance program covers installations in the states of CT, DE, IN, KY, MD, MA, MI, NH, NJ, NY, NC, OH, PA, RI, VT, VA, and WV and in DC. This program includes monitoring specifically for *Aedes albopictus*.
2. Summary of Ovitrapping during 1991, Occupational and Environmental Health Directorate, Armstrong Laboratory, Brooks Air Force Base, TX, and information from 1100th Medical squadron/SGPM, Bolling AFB, DC.

[b] Several species of mosquitos were identified from the total number collected. These species are not significant vectors for transmitting dengue virus. Full details are available in the source documents.

[c] Data not available. Full details available from source document